Genomic DNA from Plant

User Manual

NucleoSpin® Plant II

NucleoSpin® Plant II Midi

NucleoSpin® Plant II Maxi

Visit our website for more details! click here...

This product is distributed by Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave., Mountain View, CA 94043
www.clontech.com

For ordering information
1.800.662.2566
orders@clontech.com

For technical support
1.800.662.2566
tech@clontech.com

PT5028-1
PR113798

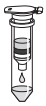
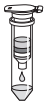
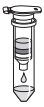
Clontech

December 2010 / Rev. 05

MACHEREY-NAGEL

Genomic DNA Purification from Plant

Protocol-at-a-glance (Rev. 05)

NucleoSpin® Plant II	Mini	Midi	Maxi
1 Homogenize samples	100 mg	400 mg	1500 mg
2 Cell lysis	400 µL **PL1** 10 µL RNase A 65°C, 10 min ***ALTERNATIVELY*** 300 µL **PL2** 10 µL RNase A 65°C, 10 min 75 µL **PL3** on ice, 5 min	1.7 mL **PL1** 25 µL RNase A 65°C, 10 min ***ALTERNATIVELY*** 1.5 mL **PL2** 25 µL RNase A 65°C, 15 min 200 µL **PL3** on ice, 5 min	6 mL **PL1** 100 µL RNase A 65°C , 10 min ***ALTERNATIVELY*** 5.3 mL **PL2** 100 µL RNase A 65°C, 20 min 700 µL **PL3** on ice, 5 min
3 Filtration / Clarification of lysate	 ≥11,000 x *g* 2 min	4,500 x *g* 10 min	4,500 x *g* 10 min
4 Adjust DNA binding conditions	450 µL PC	2.3 mL PC	10 mL PC
5 Bind DNA	 ≥11,000 x *g* 1 min	4,500 x *g* 2 min	4,500 x *g* 2 min
6 Wash and dry silica membrane	**1st** 400 µL PW1 ≥11,000 x *g* 1 min  **2nd** 700 µL PW2 ≥11,000 x *g* 1 min **3rd** 200 µL PW2 ≥11,000 x *g* 2 min	**1st** 1 mL PW1 4,500 x *g* 2 min **2nd** 3 mL PW2 4,500 x *g* 2 min **3rd** 1 mL PW2 4,500 x *g* 10 min	**1st** 4 mL PW1 4,500 x *g* 2 min **2nd** 10 mL PW2 4,500 x *g* 2 min **3rd** 2 mL PW2 4,500 x *g* 10 min
7 Elute DNA	50 µL PE 65°C, 5 min ≥11,000 x *g* 1 min Repeat elution step	200 µL PE 65°C, 5 min 4,500 x *g* 2 min Repeat elution step	1000 µL PE 65°C, 5 min 4,500 x *g* 2 min Repeat elution step

MACHEREY-NAGEL GmbH & Co. KG • Neumann-Neander-Str. 6-8 • D-52355 Düren • Germany
Tel.: +49 (0) 24 21 969 270 • www.mn-net.com • e-mail: tech-bio@mn-net.com

Table of contents

1 Components 4
- 1.1 Kit contents 4
- 1.2 Reagents, consumables, and equipment to be supplied by user 6
- 1.3 About this User Manual 6

2 Product description 7
- 2.1 The basic principle 7
- 2.2 Kit specifications 7
- 2.3 Storage of plant samples 8
- 2.4 Homogenization of plant samples 8
- 2.5 Lysis of plant samples 9
- 2.6 Elution procedures 11

3 Storage conditions and preparation of working solutions 13

4 Safety instructions – risk and safety phrases 14

5 NucleoSpin® Plant II protocols 15
- 5.1 Standard protocol for genomic DNA from plant 15
- 5.2 Support protocol for genomic DNA from fungi 19
- 5.3 Support protocol for soil, compost, dung, and animal excrements 20

6 NucleoSpin® Plant II Midi protocol 21

7 NucleoSpin® Plant II Maxi protocol 25

8 Appendix 29
- 8.1 Troubleshooting 29
- 8.2 Ordering information 31
- 8.3 Product use restriction / warranty 32

1 Components

1.1 Kit contents

	NucleoSpin® Plant II		
	10 preps	**50 preps**	**250 preps**
REF	**740770.10**	**740770.50**	**740770.250**
Lysis Buffer PL1	5 mL	25 mL	125 mL
Lysis Buffer PL2	4 mL	20 mL	100 mL
Precipitation Buffer PL3	1 mL	5 mL	25 mL
Binding Buffer PC	6 mL	30 mL	125 mL
Wash Buffer PW1	6 mL	30 mL	125 mL
Wash Buffer PW2 (Concentrate)*	6 mL	25 mL	50 mL
Elution Buffer PE**	5 mL	15 mL	30 mL
RNase A (lyophilized)*	1.5 mg	6 mg	2 x 15 mg
NucleoSpin® Filters (violet rings)	10	50	250
NucleoSpin® Plant II Columns (green rings)	10	50	250
Collection Tubes (2 mL)	20	100	500
User Manual	1	1	1

* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer PE: 5 mM Tris/HCl, pH 8.5

1.1 Kit contents *continued*

	NucleoSpin® Plant II Midi	NucleoSpin® Plant II Maxi
	20 preps	10 preps
REF	740771.20	740772.10
Lysis Buffer PL1	2 x 25 mL	75 mL
Lysis Buffer PL2	2 x 20 mL	60 mL
Precipitation Buffer PL3	5 mL	15 mL
Binding Buffer PC	2 x 30 mL	125 mL
Wash Buffer PW1	30 mL	50 mL
Wash Buffer PW2 (Concentrate)*	25 mL	50 mL
Elution Buffer PE**	15 mL	30 mL
RNase A (lyophilized)*	6 mg	10 mg
NucleoSpin® Filters L/XL (plus Collection Tubes)	20	10
NucleoSpin® Plant II Midi/Maxi Columns (plus Collection Tubes)	20	10
Collection Tubes (15 mL/50 mL)	20	10
User Manual	1	1

* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer PE: 5 mM Tris/HCl, pH 8.5

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100% ethanol

Consumables

- 1.5 mL microcentrifuge tubes **(NucleoSpin® Plant II)** or 15/50 mL tubes **(NucleoSpin® Plant II Midi / Maxi)** for elution
- Disposable tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes **(NucleoSpin® Plant II)** or an appropriate centrifuge with swing-out rotors capable of reaching 4,500 x *g* for 15 mL/50 mL tubes **(NucleoSpin® Plant II Midi / Maxi)**
- Thermal heating-block or water bath for incubation and preheating of Elution Buffer PE (to 65°C)
- Equipment for sample disruption and homogenization (see section 2.4)
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this User Manual

It is strongly recommended reading the detailed protocol sections of this User Manual if the **NucleoSpin® Plant II / Midi / Maxi** kit is used for the first time. Experienced users, however, my refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at *www.mn-net.com*.

2 Product description

2.1 The basic principle

The plant samples are homogenized by mechanical treatment. Then the DNA can be extracted with Lysis Buffers PL1 or PL2 containing chaotropic salts, denaturing agents, and detergents. Crude lysates should be cleared by centrifugation and/or filtration using the **NucleoSpin® Filters** provided with the kits in order to remove polysaccharides, contaminations, and residual cellular debris. The clear flow-through is mixed with Binding Buffer PC to create conditions for optimal binding of DNA to the silica membrane. After loading this mixture onto the spin column, contaminants are washed away using Wash Buffers PW1 and PW2. The genomic DNA can finally be eluted with low salt Elution Buffer PE (5 mM Tris/HCl, pH 8.5) or nuclease-free water and is ready-to-use for subsequent reactions.

2.2 Kit specifications

- **NucleoSpin® Plant II** kits are designed for the isolation of genomic DNA from plant tissue using two optimized lysis buffer systems based on the established CTAB and SDS methods.
- **NucleoSpin® Filters** are included for conveniently clearing the crude lysates.
- RNase A is included to remove RNA and to allow photometric quantification of pure genomic DNA.
- The optimized Binding Buffer PC and the chaotropic Wash Buffer PW1 completely remove proteins, RNA, metabolites, and other PCR inhibitors.
- The eluted DNA is ready-to-use for subsequent reactions like PCR, restriction analysis, Southern Blot etc.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Plant II	NucleoSpin® Plant II Midi	NucleoSpin® Plant II Maxi
Sample size	Up to 100 mg wet weight Up to 20 mg dry weight	Up to 400 mg wet weight Up to 80 mg dry weight	Up to 1500 mg wet weight Up to 300 mg dry weight
Typical yield	1 – 30 µg	10 – 100 µg	50 – 300 µg
Elution volume	100 µL	400 µL	2000 µL
Binding capacity	50 µg	200 µg	>500 µg
Time/prep	30 min	90 min	90 min

2.3 Storage of plant samples

Plant samples can be stored in ethanol, lyophilized, or frozen. Fresh material can be kept at 4 °C for one day but should be frozen at -20 °C for longer storage.

2.4 Homogenization of plant samples

As plant tissue is very robust, the lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include any type of commercial homogenizers (rotor-stator homogenizer) or bead mills using steel or glass beads. However, we recommend grinding with a mortar and pestle in the presence of liquid nitrogen to obtain optimal yields.

After homogenization and treatment of the sample with lysis buffer, the crude lysate can be cleared easily either with **NucleoSpin® Filters** or by centrifugation.

Methods to homogenize samples

- Grinding with mortar and pestle in the presence of liquid nitrogen: Freeze plant material in liquid nitrogen and do not let the sample thaw at any time during homogenization. Precool mortar and pestle using liquid nitrogen. Grind frozen sample thoroughly to a fine powder and refill mortar occasionally with liquid nitrogen to keep the sample frozen. Use a precooled spatula to transfer the sample in precooled tubes. Make sure no liquid nitrogen is transferred or all nitrogen has evaporated before closing the tube.

- VA steel beads (diameter: 7 mm, sample available on request): Put 4 – 5 beads and plant material into a 15 mL plastic tube (Falcon), chill the tube in liquid nitrogen and vortex for about 30 seconds (e.g., with a Multi Pulse Vortexer, Schütt Labortechnik GmbH, *www.schuett-labortechnik.de*). Repeat the chilling and vortexing procedure until the entire plant material is ground to a fine powder. Chill the tube once more and remove the beads by rolling them out gently or using a magnet. Keep the material frozen throughout the whole homogenization procedure. Do not add nitrogen to the tube since this leads to sticking and loss of plant material attached to the beads.

- Rotor-stator homogenizers are only useful to disrupt soft plants in the presence of lysis buffer. Keep homogenizer submerged at all times to reduce foaming.

2.5 Lysis of plant samples

Increasing the amount of starting material

The standard protocols of **NucleoSpin® Plant II / Midi / Maxi** kits allow processing of 10 – 1500 mg of plant material. This usually yields 1 – 300 µg of high quality DNA. However, the amount of DNA that can be expected per mg of sample depends on the size and ploidy of the genome. For example 100 mg fresh wheat with a hexaploid genome (1.7×10^{10} bp) contains 30 µg DNA, whereas the same amount of Arabidopsis with a smaller diploid genome (1.9×10^{8} bp) only yields 3 µg DNA.

Thus, it might be advantageous to process even more than the recommended sample mass (up to 5-fold) to obtain a reasonable DNA yield. However, to ensure a complete lysis, all lysis buffer volumes of protocol step 2 have to be increased proportionally and multiple loading steps are necessary. Additional lysis buffers (PL1, PL2, PL3, RNase) have to be purchased separately (see ordering information).

Choosing the optimal lysis buffer system

Plants are very heterogeneous and contain varying amounts of polyphenols, acidic components, or polysaccharides which can lead to suboptimal DNA extraction or performance in downstream applications. Therefore, we offer two different lysis buffers for optimal processing, high yields, and an excellent DNA quality with most common plant species.

The standard protocol uses Lysis Buffer PL1, which is based on the established CTAB procedure. Additionally, the SDS based Lysis Buffer PL2 is provided which requires subsequent protein precipitation by potassium acetate (Precipitation Buffer PL3). For some plant species Lysis Buffers PL1 and PL2 can be used with similar results. However, for most plant material the lysis efficiency is different due to the negative charge of SDS and the positive charge of CTAB.

Table 2 gives an overview about customer data on different plant species and the corresponding buffer system that has been tested successfully using **NucleoSpin® Plant II. Important! For a large variety of plant species both lysis buffers allow good results.**

Use the table only for a rough orientation and guideline which buffer system has already been tested. In order to find optimal lysis conditions when using a certain plant sample for the first time, it is recommended to do side-by-side preparations of one batch of homogeneously ground material with both lysis buffers.

Table 2: Plant species tested with NucleoSpin® Plant II

Plant species	Plant tissue / organ	Lysis buffer **successfully** tested	
		PL1	**PL2**
Abies alba (fir)	Needle	✔	✔
Amorphophallus titanum	Leaf	✔	Not tested
Apium graveolens (celery)	Corm	✔	✔
Arabidopsis thaliana	Leaf	✔	✔
Boreava orientalis	Leaf, herbarium sample	✔	✔
Cleisostoma racemiferum	Inflorescence rachis, silica-gel dried	✔	Not tested
Doritis pulcherrima	Leaf, silica-gel dried	✔	Not tested
Eichornia azurea	Leaf	✔	Not tested
Encephalartos natalensis	Leaf	✔	Not tested
Galium aparine	Leaf	✔	✔
Hordeum sp. (barley)	Leaf	✔	✔
Isatis kotschyana	Leaf, herbarium sample	✔	✔
Laurus azorica (laurel)	Leaf	✔	Not tested
Lupinus sp. (lupin)	Leaf	✔	✔
Lycopersicon esculentum (tomato)	Stem	✔	✔
Myagrum perfoliatum	Leaf, herbarium sample	✔	✔
Oryza sativa (rice)	Leaf	✔	✔
Persea feru./caerulea	Leaf	✔	Not tested
Pteridium sp.	Leaf	✔	Not tested
Pterocarya fraxiniofolia	Leaf	✔	Not tested
Rosa sp. (rose)	Leaf	✔	✔
Rubus fruticosus (blackberry)	Leaf	✔	✔
Sameraria nummularia	Leaf, herbarium sample	✔	✔
Secale sp. (rye)	Leaf	✔	✔
Stereochilus sp.	Leaf, silica-gel dried	✔	Not tested
Tauscheria lasiocarpum	Leaf, herbarium sample	✔	✔
Trachycarpus takil	Leaf	✔	Not tested
Trichoglottis sp.	Leaf, silica-gel dried	✔	Not tested
Triticum aestivum (wheat)	Leaf	✔	✔
Vigna radiata (mung bean)	Root	✔	✔
Zea mays (maize)	Leaf	✔	✔
Zea mays (maize)	Grain, dried, ground coarsely	✔	✔
Fungal mycel (not specified)		✔	Not tested
Green algae (not specified)		✔	Not tested

2.6 Elution procedures

The following graphs show DNA yields (solid line) and the resulting DNA concentrations (dotted line) in dependence on elution buffer volume. Elution is done with either one elution step (A) or two subsequent elution steps with the indicated elution buffer volume each (B).

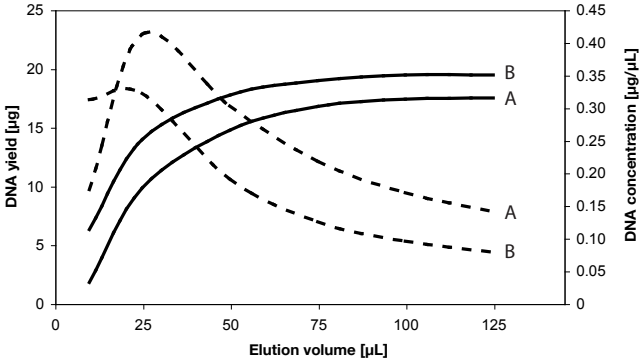


Figure 1: NucleoSpin® Plant II elution profile

Genomic DNA from 100 mg fresh wheat leaves was purified and eluted once (A) or twice (B) with 10 – 125 µL Buffer PE. Resulting yield and concentration is shown as solid and dotted lines, respectively.

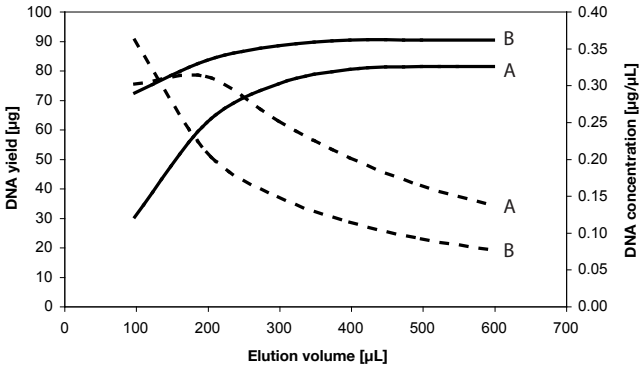


Figure 2: NucleoSpin® Plant II Midi elution profile

Genomic DNA from 400 mg fresh wheat leaves was purified and eluted once (A) or twice (B) with 100 – 600 µL Buffer PE. Resulting yield and concentration is shown as solid and dotted lines, respectively.

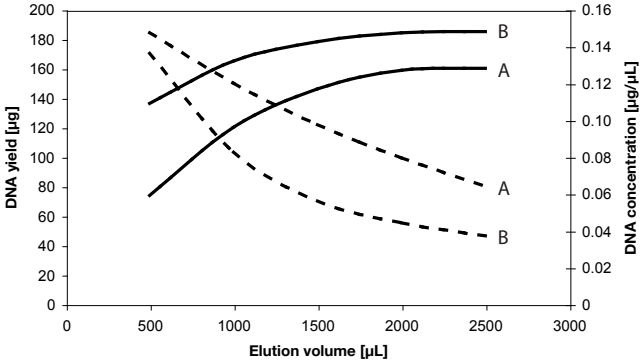


Figure 3: NucleoSpin® Plant II Maxi elution profile

Genomic DNA from 1000 mg fresh wheat leaves was purified and eluted once (A) or twice (B) with 500 – 2500 µL Buffer PE. Resulting yield and concentration is shown as solid and dotted lines, respectively.

A two-fold elution generally yields more DNA than just one elution with the same total buffer volume. This is most important for small buffer volumes. However, large volumes or eluting two times results in a lower DNA concentration.

The standard elution procedure is already optimized to yield 80 – 90% by eluting two-fold at elevated temperatures. However, if even higher yields, a higher concentration, or maximum speed is required, the elution procedure can be adapted:

Table 3: Elution parameters

Procedure (% of exp. yield)	NucleoSpin® Plant II	NucleoSpin® Plant II Midi	NucleoSpin® Plant II Maxi
Standard elution (85 – 90%)	50 µL + 50 µL 65°C, 5 min	200 µL + 200 µL 65°C, 5 min	1000 µL + 1000 µL 65°C, 5 min
Maximum yield (95 – 100%)	100 µL + 100 µL 65°C, 5 min	400 µL + 400 µL 65°C, 5 min	2000 µL + 2000 µL 65°C, 5 min
High concentration (75%)	25 µL + 25 µL 65°C, 5 min	100 µL + 100 µL 65°C, 5 min	500 µL + 500 µL 65°C, 5 min
Fast elution (60 – 70%)	100 µL RT/65°C, 1 – 5 min	400 µL RT/65°C, 1 – 5 min	2000 µL RT/65°C, 1 – 5 min

3 Storage conditions and preparation of working solutions

Attention:

Buffers PL1, PL2, PC, and PW1 contain guanidine hydrochloride and/or detergents like CTAB or SDS! Wear gloves and goggles!

All kit components can be stored at room temperature (18 – 25°C) and are stable for at least one year.

Before starting any **NucleoSpin® Plant II** protocol prepare the following:

- **Lysis Buffer PL2:** Check for precipitated SDS especially after storage at temperatures below 20°C. If necessary incubate the bottle for several minutes at 30 – 40°C and mix well until the precipitate is re-dissolved completely.
- **Wash Buffer PW2:** Add the given volume of ethanol (96 – 100%) as indicated on the bottle or in the table below to **Buffer PW2 Concentrate** before first use. Mark the label of the bottle to indicate that the ethanol is added. Buffer PW2 at is stable at room temperature (18 – 25°C) for at least one year.
- **RNase A:** Add the given volume of water as indicated on the vial and in the table below to lyophilized **RNase A**. Store the **RNase A solution** at **4 °C** for **up to 3 months**. For longer storage (up to 1 year), the RNase A solution should be divided into small aliquots and stored at **-20 °C**.

	NucleoSpin® Plant II		
	10 preps	**50 preps**	**250 preps**
REF	**74070.10**	**740770.50**	**740770.250**
Wash Buffer PW2 (Concentrate)	6 mL add 24 mL ethanol	25 mL add 100 mL ethanol	50 mL add 200 mL ethanol
RNase A	1.5 mg dissolve in 150 µL H_2O	6 mg dissolve in 600 µL H_2O	2 x 15 mg dissolve in 1500 µL H_2O each

	NucleoSpin® Plant II Midi	**NucleoSpin® Plant II Maxi**
	20 preps	**10 preps**
REF	**740771.20**	**740772.10**
Wash Buffer PW2 (Concentrate)	25 mL add 100 mL ethanol	50 mL add 200 mL ethanol
RNase A	6 mg dissolve in 600 µL H_2O	10 mg dissolve in 1100 µL H_2O

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® Plant II** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
PC	Guanidine hydrochloride + ethanol <40%	Xn**	Flammable - Harmful if swallowed - Irritating to eyes and skin	R 10-22-36/38	S 7-16
PW1	Guanidine hydrochloride + isopropanol < 25%	Xn**	Flammable - Harmful if swallowed - Irritating to eyes and skin	R 10-22-36/38	S 7-16-25
RNase A	RNase A, lyophilized	Xn	May cause sensitization by inhalation and skin contact	R 42/43	S 22-24

Risk phrases

R 10	Flammable
R 22	Harmful if swallowed
R 36/38	Irritating to eyes and skin
R 42/43	May cause sensitization by inhalation and skin contact

Safety phrases

S 7	Keep container tightly closed
S 16	Keep away from source of ignition - No Smoking!
S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 25	Avoid contact with the eyes

* Hazard labeling not neccessary if quantity per bottle below 25 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

** Hazard labeling not neccessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

5 NucleoSpin® Plant II protocols

5.1 Standard protocol for genomic DNA from plant

Before starting the preparation:

- Check if Wash Buffer PW2 and RNase A were prepared according to section 3.
- Preheat Elution Buffer PE to 65 °C.

Note: The NucleoSpin® Plant II kits include two different lysis buffers for optimal results with most common plant species. Please refer to section 2.5 for choosing the optimal lysis buffer system for your individual plant sample and for information on how to process even more sample material than recommended in the following protocol.

1 Homogenize sample

Homogenize up to 100 mg wet weight or up to 20 mg dry weight (lyophilized) plant material (for homogenization methods see section 2.4).

Homogenize samples

Proceed with cell lysis using **Buffer PL1** (step 2a) or alternatively **Buffer PL2** (step 2b).

2a Cell lysis using Buffer PL1

Transfer the resulting powder to a new tube and add **400 µL Buffer PL1**. Vortex the mixture thoroughly.

+ 400 µL PL1

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL1** can be added. Note that the volumes of **RNase A** (step 2a) and **Buffer PC** (step 4) have to be increased proportionally.*

Add **10 µL RNase A** solution and mix sample thoroughly.

+ 10 µL RNase A

Incubate the suspension for **10 min** at **65°C**.

65 °C 10 min

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with step 3.

2b Cell lysis using Buffer PL2

+ 300 µL PL2

Transfer the resulting powder to a new tube and add **300 µL Buffer PL2**. Vortex the mixture thoroughly.

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL2** can be added. Note that the volumes of **RNase A, Buffer PL3** (step 2b), **and Buffer PC** (step 4) have to be increased proportionally.*

+ 10 µL RNase A

Add **10 µL RNase A** solution and mix sample thoroughly.

65 °C
10 min

Incubate the suspension for **10 min at 65 °C**.

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

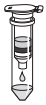
+ 75 µL PL3
on ice
5 min

Add **75 µL Buffer PL3**, mix thoroughly and incubate for **5 minutes on ice** to precipitate SDS completely.

Proceed with step 3.

3 Filtration / Clarification of crude lysate

Place a **NucleoSpin® Filter** (violet ring) into a new Collection Tube (2 mL) and load the lysate onto the column. Centrifuge for **2 min** at **11,000 x *g***, collect the clear flow-through and discard the NucleoSpin® Filter.



If not all liquid has passed the filter, repeat the centrifugation step.

11,000 x *g*
2 min

If a pellet is visible in the flow-through, transfer the clear supernatant to a new 1.5 mL microcentrifuge tube (not provided).

Alternatively, centrifuge the crude lysate for 5 min at 11,000 x g and transfer the supernatant to a new tube or pass the precleared supernatant through the NucleoSpin® Filter to remove solid particles completely.

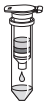
4 Adjust DNA binding conditions

+ 450 µL PC

Add **450 µL Buffer PC** and mix thoroughly by pipetting up and down (5 times) or by vortexing.

5 Bind DNA

Place a **NucleoSpin® Plant II Column** (green ring) into a new Collection Tube (2 mL) and load a maximum of 700 µL of the sample.



Load lysate

Centrifuge for **1 min** at **11,000 x *g*** and discard the flow-through.

11,000 x *g*
1 min

The maximum loading capacity of the NucleoSpin® Plant II Column is 700 µL. For higher sample volumes repeat the loading step.

6 Wash and dry silica membrane

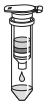
+ 400 µL
PW1

1st wash

Add **400 µL Buffer PW1** to the NucleoSpin® Plant II Column. Centrifuge for **1 min** at **11,000 x *g*** and discard flow-through.

11,000 x *g*
1 min

Note: Although washing with Buffer PW1 increases purity it can in some cases slightly reduce the final yield.



+ 700 µL
PW2

2nd wash

Add **700 µL Buffer PW2** to the NucleoSpin® Plant II Column. Centrifuge for **1 min** at **11,000 x *g*** and discard flow-through.

11,000 x *g*
1 min

+ 200 µL
PW2

3rd wash

Add another **200 µL Buffer PW2** to the NucleoSpin® Plant II Column. Centrifuge for **2 min** at **11,000 x *g*** in order to remove wash buffer and dry the silica membrane completely.

11,000 x *g*
2 min

7 Elute DNA

+ 50 µL PE
65 °C
5 min

Place the NucleoSpin® Plant II Column into a new 1.5 mL microcentrifuge tube (not provided).

11,000 x *g*
1 min

Pipette **50 µL Buffer PE (65°C)** onto the membrane. Incubate the NucleoSpin® Plant II Column for **5 min** at **65°C**. Centrifuge for **1 min** at **11,000 x *g*** to elute the DNA.

+ 50 µL PE
65 °C
5 min

Repeat this step with another **50 µL Buffer PE (65°C)** and elute into the same tube.

11,000 x *g*
1 min

Note: To achieve maximum yield or higher concentrations refer to section 2.6 for alternative elution procedures.

5.2 Support protocol for genomic DNA from fungi

Attention: Additional reagents and equipment necessary!

- Ethanol (96 – 100%)
- Chloroform
- Micro pistill
- Siliconized glass beads or sea sand

1 Homogenize sample

Wash **50 – 200 mg mycelium** (fresh weight) or material from a fruiting body of macro fungi in **ethanol**. Mycelium can be obtained from a liquid culture or scraped off (with or without agar) from the surface of a solid medium.

Cover sample completely with **ethanol** and mix carefully. Short washing in ethanol is sufficient in most cases, although incubation overnight sometimes increases DNA yield. (Long-term storage in ethanol is also possible).

Remove ethanol by pipetting and squeezing the mycelium.

2 Cell lysis

Place the sample into a 1.5 mL microcentrifuge tube (not provided). Add **150 mg siliconized glass beads** or **sea sand** and **200 µL Buffer PL1**. Homogenize sample using a micro pistil and vortex regularly. Add additional **100 µL Buffer PL1** and continue to homogenize the sample.

*Note: If the sample can not be handled easily because e.g. the sample material is soaking up too much buffer, additional **Buffer PL1** can be added. Note that the volume of **Buffer PC** (step 4) has to be increased proportionally.*

*Optional: If the sample is rich in RNA or protein, we recommend adding **10 µL RNase A and/or Proteinase K** (5 – 10 mg/mL stock solution, see ordering information), respectively, to the **PL1 lysis solution** in order to minimize contaminants.*

Incubate for **10 min** at **65 °C**.

Add **100 µL chloroform**. Vortex for **10 s** and separate phases by centrifugation for **15 min** at **20,000 x *g***. Pipette the top aqueous layer into a new 1.5 mL microcentrifuge tube (not provided).

For some fungi it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with section 5.1, step 3.

5.3 Support protocol for soil, compost, dung, and animal excrements

Attention: Additional equipment necessary!

- Bead mill (e.g., Pulverisette 0, Fritsch – Idar-Oberstein) or mortar and pestle
- Sea sand (siliconized)
- Extraction buffer: 2 M NaCl, 20 mM EDTA, 100 mM Tris/HCl, 2% (w/v) CTAB, 2% (w/v) Polyvinylpyrrolidon (MW 40,000), pH 8.0

1 Homogenize sample

Weigh **5 g soil** or **2 g dung** into a petri dish. Add extraction buffer until the sample is completely soaked. Heat the sample in a **microwave oven** (400 W) for a few seconds until the extraction buffer is foaming.

Extraction buffer may be added to keep the sample in a slushy state.

2 Cell lysis

Transfer sample into a bead mill or mortar. Add **0.5 mL sea sand** and disrupt the sample.

3 Filtration / Clarification of lysate

Transfer the homogenized sample into a centrifuge tube (e.g., Sorvall SS34) and centrifuge for **10 min** at **5,000 x *g***. Pipette **300 µL** of the clear supernatant into a new 1.5 mL microcentrifuge tube (not provided).

Proceed with section 5.1, step 3.

6 NucleoSpin® Plant II Midi protocol

Before starting the preparation:

- Check if Wash Buffer PW2 and RNase A were prepared according to section 3.
- Preheat Elution Buffer PE to 65 °C.
- A centrifuge with a swing-out rotor and appropriate buckets capable of reaching 4,500 x *g* is required.

Note: The NucleoSpin® Plant II Midi kits include two different lysis buffers for optimal results with most common plant species. Please refer to section 2.5 for choosing the optimal lysis buffer system for your individual plant sample and for information on how to process even more sample material than recommended in the following protocol.

1 Homogenize sample

Homogenize up to 400 mg wet weight or up to 80 mg dry weight (lyophilized) plant material (for homogenization methods see section 2.4).

Homogenize samples

Proceed with cell lysis using **Buffer PL1** (step 2a) or alternatively **Buffer PL2** (step 2b).

2a Cell lysis using Buffer PL1

Transfer the resulting powder to a new tube and add **1.7 mL Buffer PL1**. Vortex the mixture thoroughly.

+ 1.7 mL PL1

*Note: If the sample can not be resuspended easily because e.g. the plant powder is soaking up too much buffer, additional **Buffer PL1** can be added. Note that the volumes of **RNase A** (step 2a) and **Buffer PC** (step 4) have to be increased proportionally.*

Add **25 µL RNase A** solution and mix sample thoroughly.

+ 25 µL RNase A

Incubate the suspension for **15 min** at **65°C**.

65 °C 10 min

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with step 3.

2b Cell lysis using Buffer PL2

Transfer the resulting powder to a new tube and add **1.5 mL Buffer PL2**. Vortex the mixture thoroughly.

+ 1.5 mL PL2

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL2** can be added. Note that the volumes of **RNase A, Buffer PL3** (step 2b), and **Buffer PC** (step 4) have to be increased proportionally.*

+ 25 µL RNase A

Add **25 µL RNase A** solution and mix sample thoroughly.

Incubate the suspension for **15 min** at **65°C**.

65 °C
10 min

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

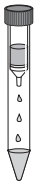
Add **200 µL Buffer PL3**, mix thoroughly and incubate for **5 min on ice** to precipitate SDS completely.

+ 200 µL PL3
on ice
5 min

Proceed with step 3.

3 Filtration / Clarification of crude lysate

Transfer the lysate to a **NucleoSpin® Filter L**. Centrifuge for **10 min** at **4,500 x *g***, collect the clear flow-through and discard the NucleoSpin® Filter L.



If not all liquid has passed the filter, repeat the centrifugation step.

If a pellet is visible in the flow-through, transfer the clear supernatant to a new 15 mL microcentrifuge tube (not provided).

4,500 x *g*
10 min

Alternatively, centrifuge the crude lysate for 5 min at 4,500 x g and transfer the supernatant to a new tube or pass the precleared supernatant through the NucleoSpin® Filter L to remove solid particles completely.

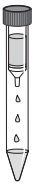
4 Adjust DNA binding conditions

Add **2.3 mL Buffer PC** to the cleared lysate and mix immediately by vortexing for **30 s**.

+ 2.3 mL PC

Vortex 30 s

5 Bind DNA



Load sample

Load sample on a **NucleoSpin® Plant II Midi Column.**

Centrifuge for **2 min** at **4,500 x *g*** and discard the flow-through.

The maximum loading capacity of the NucleoSpin® Plant II Midi Column is 5 mL. For higher sample volumes repeat the loading step.

4,500 x *g*
2 min

6 Wash and dry silica membrane

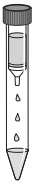
1st wash

+ 1 mL PW1

Add **1 mL Buffer PW1** to the NucleoSpin® Plant II Midi Column. Centrifuge for **2 min** at **4,500 x *g*** and discard flow-through.

4,500 x *g*
2 min

Note: Although washing with Buffer PW1 increases purity it can in some cases slightly reduce the final yield.



2nd wash

+ 3 mL PW2

Add **3 mL Buffer PW2** to the NucleoSpin® Plant II Midi Column. Centrifuge for **2 min** at **4,500 x *g*** and discard flow-through.

4,500 x *g*
2 min

3rd wash

+ 1 mL PW2

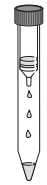
Add another **1 mL Buffer PW2** to the NucleoSpin® Plant II Midi Column. Centrifuge for **10 min** at **4,500 x *g*** in order to remove wash buffer and dry the silica membrane completely.

4,500 x *g*
10 min

7 Elute DNA

Place the NucleoSpin® Plant II Midi Column into a new Collection Tube (15 mL)

Pipette **200 µL Buffer PE (65°C)** onto the membrane. Incubate the NucleoSpin® Plant II Midi Column for **5 min** at **65°C**. Centrifuge for **2 min** at **4,500 x *g*** to elute the DNA.



Repeat this step with another **200 µL Buffer PE (65°C)** and elute into the same tube.

Note: To achieve maximum yield or higher concentrations refer to section 2.6 for alternative elution procedures.

+ 200 µL PE
65 °C
5 min

4,500 x *g*
2 min

+ 200 µL PE
65 °C
5 min

4,500 x *g*
2 min

7 NucleoSpin® Plant II Maxi protocol

Before starting the preparation:

- Check if Wash Buffer PW2 and RNase A were prepared according to section 3.
- Preheat Elution Buffer PE to 65°C.
- A centrifuge with a swing-out rotor and appropriate buckets capable of reaching 4,500 x *g* is required.

Note: The NucleoSpin® Plant II Maxi kits include two different lysis buffers for optimal results with most common plant species. Please refer to section 2.5 for choosing the optimal lysis buffer system for your individual plant sample and for information on how to process even more sample material than recommended in the following protocol.

1 Homogenize sample

Homogenize up to 1500 mg wet weight or up to 300 mg dry weight (lyophilized) plant material (for homogenization methods see section 2.4).

Homogenize samples

Proceed with cell lysis using **Buffer PL1** (step 2a) or alternatively **Buffer PL2** (step 2b).

2a Cell lysis using Buffer PL1

Transfer the resulting powder to a new tube and add **6 mL Buffer PL1**. Vortex the mixture thoroughly.

+ 6 mL PL1

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL1** can be added. Note that the volumes of **RNase A** (step 2a) and **Buffer PC** (step 4) have to be increased proportionally.*

Add **100 µL RNase A** solution and mix sample thoroughly.

+ 100 µL RNase A

Incubate the suspension for **20 min** at **65°C**.

65 °C 10 min

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with step 3.

2b **Cell lysis using Buffer PL2**

Transfer the resulting powder to a new tube and add **5.3 mL Buffer PL2**. Vortex the mixture thoroughly.

+ 5.3 mL PL2

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL2** can be added. Note that the volumes of **RNase A**, **Buffer PL3** (step 2b), and **Buffer PC** (step 4) have to be increased proportionally.*

+ 100 µL RNase A

Add **100 µL RNase A** solution and mix sample thoroughly.

Incubate the suspension for **20 min** at **65°C**.

65 °C
10 min

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Add **700 µL Buffer PL3**, mix thoroughly and incubate for **5 min on ice** to precipitate SDS completely.

+ 700 µL PL3
on ice
5 min

Proceed with step 3.

3 **Filtration / Clarification of crude lysate**

Transfer the lysate to a **NucleoSpin® Filter XL**. Centrifuge for **10 min** at **4,500 x *g***, collect the clear flow-through and discard the NucleoSpin® Filter XL.

If not all liquid has passed the filter, repeat the centrifugation step.

If a pellet is visible in the flow-through, transfer the clear supernatant to a new 50 mL microcentrifuge tube (not provided).

4,500 x *g*
10 min

Alternatively, centrifuge the crude lysate for 5 min at 4,500 x g and transfer the supernatant to a new tube or pass the precleared supernatant through the NucleoSpin® Filter XL to remove solid particles completely.

4 **Adjust DNA binding conditions**

Add **10 mL Buffer PC** to the cleared lysate and mix immediately by vortexing for **30 s**.

+ 10 mL PC

Vortex 30 s

5 Bind DNA

Load sample

Load sample on a **NucleoSpin® Plant II Maxi Column**

Centrifuge for **2 min** at **4,500 x *g*** and discard the flow-through.

4,500 x *g*
2 min

The maximum loading capacity of the NucleoSpin® Plant II Maxi Column is 15 mL. For higher sample volumes repeat the loading step.

6 Wash and dry silica membrane

1st wash

+ 4 mL PW1

Add **4 mL Buffer PW1** to the NucleoSpin® Plant II Maxi Column. Centrifuge for 2 min at 4,500 x g and discard flow-through.

4,500 x *g*
2 min

Note: Although washing with Buffer PW1 increases purity it can in some cases slightly reduce the final yield.

+ 10 mL PW2

2nd wash

4,500 x *g*
2 min

Add **10 mL Buffer PW2** to the NucleoSpin® Plant II Maxi Column. Centrifuge for **2 min** at **4,500 x *g*** and discard flow-through.

+ 2 mL PW2

3rd wash

4,500 x *g*
10 min

Add another **2 mL Buffer PW2** to the NucleoSpin® Plant II Maxi Column. Centrifuge for **10 min** at **4,500 x *g*** in order to remove wash buffer and dry the silica membrane completely.

7 Elute DNA

Place the NucleoSpin® Plant II Maxi Column into a new Collection Tube (50 mL)

Pipette **1000 µL Buffer PE (65°C)** onto the membrane. Incubate the NucleoSpin® Plant II Maxi Column for **5 min** at **65°C**. Centrifuge for **2 min** at **4,500 x *g*** to elute the DNA.

+ 1000 µL PE
65 °C
5 min

4,500 x *g*
2 min

Repeat this step with another **1000 µL Buffer PE (65°C)** and elute into the same tube.

+ 1000 µL PE
65 °C
5 min

4,500 x *g*
2 min

Note: To achieve maximum yield or higher concentrations refer to section 2.6 for alternative elution procedures.

8 Appendix

8.1 Troubleshooting

Problem	Possible cause and suggestions
DNA yield is low	*Homogenization of plant material was not sufficient* • For most species we recommend grinding with steel beads or mortar and pestle (see section 2.4). For disruption of the cell wall it is important to homogenize the plant material thoroughly until the sample is ground to a fine powder. • Instead of freezing in liquid nitrogen the sample can also be lyophilized and easily ground at room temperature. *Suboptimal lysis buffer was used* • Lysis efficiencies of Buffer PL1 (CTAB) and Buffer PL2 (SDS) are different and depend on the plant species. Try both buffers in a side-by-side purification to find the best detergent system to lyse your plant material. *Suboptimal lysis buffer volume was used* • Cell lysis might be insufficient and too much DNA might get lost during lysate clarification if e.g. dry material soaks up too much lysis buffer. Use more lysis buffer and increase the volume of Binding Buffer PC proportionally. *Suboptimal binding buffer volume was used* • Increase Binding Buffer PC proportionally if more lysis buffer was used. *Extraction of DNA from plant material during lysis was insufficient* • Increase incubation time in lysis buffer (up to overnight). *Suboptimal Elution* • The DNA can either be eluted in higher volumes or by repeating the elution step up to three times. Incubate NucleoSpin® Plant II Column with elution buffer at 65°C for at least 5 minutes. • Also check the pH of the elution buffer, which should be in the range of pH 8.0 – 8.5. To ensure correct pH, use supplied Elution Buffer PE (5 mM Tris/HCl, pH 8.5).

Problem	Possible cause and suggestions
NucleoSpin® Filter or NucleoSpin® Plant II Column is clogged	*Sample was too viscous due to too much sample material or material carry-over.* • Centrifuge large amounts of sample material before loading it onto the NucleoSpin® Filter or Filter L/XL. • Make sure the cleared lysate is absolutely free of resuspended matter before loading it onto the NucleoSpin® Plant II or Plant II Midi/Maxi Column. • Increase centrifugation speed and time. • Use more Lysis Buffer PL1 or PL2.
DNA is degraded	*Sample was contaminated with DNase* • If another elution buffer than Buffer PE is used, make sure it is free of DNase activity, for example, by addition of 1 mM EDTA or heating the buffer to 70 °C for 10 min. *Centrifugation speed was too high* • Centrifuge at a maximum speed of 11,000 x *g*. Higher velocities may lead to shearing of the DNA.
DNA quality is low	*Elution buffer contains EDTA* • EDTA may disturb subsequent reactions. Use water or the supplied Elution Buffer PE (5 mM Tris/HCl, pH 8.5) for elution. *Salt or ethanol carry-over* • Make sure the last two wash steps were done with Wash Buffer PW2 and the membrane was dried according to the protocol.

8.2 Ordering information

Product	REF	Pack of
NucleoSpin® Plant II	740770.10/.50/.250	10/50/250 preps
NucleoSpin® Plant II Midi	740771.20	20 preps
NucleoSpin® Plant II Maxi	740772.10	10 preps
Buffer PL1	740918	125 mL
Buffer Set PL2/PL3 (100 mL Buffer PL2 + 25 mL Buffer PL3)	740919	1 set
Buffer PC	740937	125 mL
Buffer PW1	740938	125 mL
Buffer PW2 Concentrate (for 250 mL Buffer PW2)	740939	50 mL
RNase A	740505.50 740505	50 mg 100 mg
Proteinase K	740506	100 mg
NucleoSpin® Filters (for filtration of cell homogenates)	740606	50
Collection Tubes (2 mL)	740600	1000

8.3 Product use restriction/warranty

NucleoSpin® Plant II/Midi/Maxi kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or

components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Last updated: 07/2010, Rev. 03

Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 (0) 24 21 969 270
e-mail: tech-bio@mn-net.com